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Miniaturized Bioengineered Models for Preterm Fetal Membrane Healing

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Mini-Summary

What does this study add to current knowledge?

- Current ex vivo fetal membrane (FM) healing models rely on term FMs. Nonetheless, the use of such models is not possible with midterm biopsies due to their small size. Our miniaturized synthetic hydrogel-based model enables the study of FM healing-promoting signals and biomaterials in three-dimensional cultures. The combination of defined biomaterials with small biopsies makes this model exceptionally suitable for the testing of preterm FM healing responses and the screening of new factors with an increased throughput.

What are the main clinical implications?

- Preterm deliveries related to iatrogenic preterm rupture of the FMs after fetal interventions are still an unsolved problem in obstetrics. Engineered biomaterials with defined in vivo stability and healing-promoting functions hold great promise for the preventive closure of FM defects immediately after surgical interventions. To advance the development of such biomaterials for the prevention of preterm birth, models that enable the testing of signals in relevant FM tissues with a sufficient large throughput are key.

Keywords
Amnion · Ex vivo models · Fetal membranes · Iatrogenic preterm prelabor rupture of fetal membranes · Preterm delivery

Abstract

Introduction: The reason for the absence of fetal membrane (FM) healing after a fetoscopic intervention is still unknown. We hypothesize that the lack of robust miniaturized models to study preterm FM functions is currently hampering the development of new treatments for FM healing. Specifically, miniaturized models to study preterm FM healing with minimal amounts of tissue are currently lacking. ***Methods:*** In this study, we collected FMs from planned cesarean deliveries and developed different ex vivo models with an engineered biomaterial to study FM healing. Then, the effect of platelet-derived growth factor BB (PDGF-BB) on the migration of cells from preterm and term FMs was evaluated. ***Results:*** FMs could be viably cultured ex vivo for 14 days. In a model of punctured FMs, migration of cells into FM defects was less pronounced than migration out of the tissue into the biomaterial. In a miniaturized model of preterm cell migration, PDGF-BB promoted migration of preterm amnion cells into

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the biomaterial. ***Discussion and Conclusion:*** By using a novel miniaturized model of preterm tissue, we here successfully demonstrate that PDGF-BB can promote preterm FM cell migration of microtissues encapsulated in a three-dimensional environment.

Introduction

Minimally invasive prenatal interventions which aim to ameliorate fetal developmental defects have become a clinical reality and are currently performed for a variety of life-threatening complications [1]. Despite their numerous benefits, they are associated with a significant risk of preterm birth, which can lead to several consequences such as intellectual impairment, chronic lung disease, cerebral palsy, blindness, and deafness [2, 3]. Iatrogenic preterm prelabor rupture of the fetal membranes (iPPROM) has been reported to happen in 30% of the cases after a fetoscopic intervention on average [4, 5], although rates as high as 60–100% have been reported [4]. Recently, the potential reasons for iPPROM have been thoroughly reviewed [6] and include postoperative chorioamniotic membrane separation [7–9] and tissue damage at the port site [10] leading to a leakage of amniotic fluid and weakened mechanical properties of the fetal membranes (FMs) [11]. The reported inability of the FMs to spontaneously heal [10, 12] has been attributed to their poor vascularization [13, 14] and might play a significant role in the risk for iPPROM. Therefore, treatments that promote the healing of FMs could successfully contribute to pregnancy prolongation and thus lower the complications associated with preterm birth. Specifically, strategies tailored toward the healing of the amnion should be developed, since it is the stronger membrane and could be responsible for restoring FM integrity.

In line with the absence of healing of the native FMs after fetoscopies [10, 12], ex vivo and in vivo studies have reported a lack of spontaneous FM healing [15–17]. In initial attempts to search for healing-promoting signals, two-dimensional (2D) in vitro scratch assays demonstrated the capacity of amnion-derived cells to migrate in the presence of stimuli [18–20]. However, to translate such findings to clinically relevant applications, bridging the FM defect with a provisional material is necessary to enable its closure and facilitate the migration of FM cells into the defect. Along these lines, several naturally occurring biomaterials have been employed as ex vivo platforms to study FM healing signals and understand FM cell migration [21]. However, the stability of these provisional healing matrices was insufficient for in vivo applications [22, 23]. For this reason, synthetic biomaterials with defined mechanical properties, defined and tailorable proteolytic degradability, cell adhesion, and growth factor (GF)-binding properties have become good alternatives for the study of healing-inducing queues. For instance, we have earlier shown that transglutaminase factor XIII crosslinked poly(ethylene glycol) (TG-PEG) hydrogels that are degradable by matrix metalloproteinase 1 and contain the cell adhesion peptide RGD are suitable substrates for the three-dimensional (3D) culture of amnion cells and tissues [24]. In that study, the ability of fibroblast GF 2 (FGF-2), epidermal GF (EGF), and platelet-derived GF BB (PDGF-BB) to stimulate FM cells to migrate outside of term FM pieces into the biomaterial was demonstrated. More specifically, PDGF-BB was the most potent factor for promoting amnion-derived cell migration and proliferation.

Although promising, all of the abovementioned ex vivo models have been developed with term FMs, which might not reflect the biology of preterm FMs. Thanks to the routinely performed surgeries to treat *spina bifida* patients in utero, now precious midterm FM samples are becoming available. However, the very small tissue fragments that can be collected from such preterm patients make the development of robust miniaturized models crucial. In this study, we systematically develop ex vivo models to assess amnion cell viability and migration in a synthetic, well-defined TG-PEG hydrogel. By studying different 3D models, we show how the specific configurations can influence the healing behavior of the amnion. Finally, a standardized method applicable to very small biopsies is used to study the mobilization of cells from preterm amnion tissues.

Materials and Methods

Tissue Collection

This study protocol was reviewed and approved by the Ethical Board of the Kanton of Zurich, approval number Stv07/2007 for term FMs and approval number PB 2020_00066 for preterm FMs. Written informed consent to participate was obtained from all participants involved in this study. FMs from term pregnancies (N = 12) were collected from cesarean sections (37–39 gestational weeks [GW]), and 3 FMs were collected from preterm pregnancies (21–31 GW: one spina bifida surgery, one induced abruption without infection, and one planned cesarean section). Applied exclusion criteria were infections, premature rupture of the FMs, and chromosomal abnormalities. The FMs were cut about 2 cm from the placental disk and outside the zone of altered morphology and

were washed for at least 20 min at 37 °C with phosphate-buffered saline (PBS) solution (Gibco) in order to remove blood and cell debris. For experiments with only amnion, the chorion and the amnion were mechanically separated by blunt dissection.

PEG Precursor Synthesis

TG-PEG hydrogels were synthesized as previously described [25, 26]. Briefly, star-shaped 8-arm PEG with pending vinylsulfone groups (40 kDa, NOF) was reacted in 0.5 M triethanolamine (pH 8.0) with a 1.2 M excess of Gln (Gln; H-NQEQVSPL-ERCG-NH_2; Bachem) or Lys (Lys-$MMP_{degradable}$; Ac-FKGG-CPQGIWGQ-ERCG-NH_2; Bachem) peptides. The resulting 8-arm PEG-Gln and 8-arm PEG-$MMP_{degradable}$-Lys precursors were extensively dialyzed against ddH_2O, lyophilized, and stored at –20 °C until use.

TG-PEG Hydrogel Formation and ex vivo Models

Dry 8-arm PEG-Gln and 8-arm PEG-$MMP_{degradable}$-Lys precursors were dissolved in Tris buffer (50 mM, pH 7.6) and used to form stoichiometrically balanced (1:1) PEG precursor solutions, which were complemented with 50 μM Gln-RGD (Ac-FKGG-RGDSPG-NH_2) and 50 mM $CaCl_2$. Covalent crosslinking was achieved by adding 10 U/mL of thrombin-activated FXIII (FXIII; Fibrogammin P, CSL Behring) after vortexing.

Punctured FM Model

Approximately 0.5 × 0.5 cm FM pieces were cut and punched either with 3-mm biopsy punch or with a 10-Fr trocar. For the punctured biopsies, a 3-mm biopsy tissue was punctured with a 1-mm biopsy punch. The FMs were transferred to a hydrophobic silicone plate, and TG-PEG was pipetted into the punctured area just after addition of activated FXIII (FXIIIa). FMs were incubated for 10 min at room temperature (RT) to allow TG-PEG polymerization and then released into tissue culture plates and covered medium (minimum essential medium alpha [MEMa; Gibco] supplemented with 10% [vol/vol] fetal bovine serum [Gibco] and 100 U/mL penicillin [Gibco] and 100 μg/mL streptomycin [Gibco]; 1% P/S). Samples were cultured at 37 °C in a humidified incubator with 5% CO_2, and medium was exchanged every 2–3 days. When applicable, PDGF-BB (PeproTech) was supplemented in the media at 100 ng/mL.

Miniaturized FM Model

Microtissues of 1-mm diameter were taken from the FMs using a 1-mm biopsy punch. The microtissues were then carefully added to 30 μL TG-PEG hydrogel drops on a hydrophobic glass slide just after adding FXIIIa. After 10 min of polymerization, the samples were released into tissue culture plates and cultured in MEMa, 10% FBS, 1% P/S at 37 °C in a humidified incubator with 5% CO_2. Medium was exchanged every 2–3 days, and when applicable, PDGF-BB (PeproTech) was supplemented in the media at 100 ng/mL. Brightfield images were taken with a DMI6000 B Leica microscope.

Histology

For histology, FMs were fixed 2 h in 4% formalin and then sandwiched in histological cassettes. Slides were then deparaffinized and rehydrated, and 4-μm-thick sections were stained for hematoxylin and eosin following standard protocols. Images were obtained with a Zeiss Axiovert 200M microscope.

Cell Viability Staining

Viability of FMs in culture was performed using a live/dead viability assay (Life Technologies). Tissues were washed 3 times with PBS and incubated in culture media with 4 μM calcein AM (1:1,000 dilution) and 2 μM ethidium homodimer-1 (EthD-1; 1:1,000 dilution) for 30 min at 37 °C in a humidified incubator with 5% CO_2. Samples were washed 3 times with PBS and kept at growth conditions until imaging with an inverted Leica TCS SP5 confocal laser-scanning microscope. Images were processed with Fiji from ImageJ [27, 28].

Cytoskeleton and Nuclei Staining

To stain for actin and nuclei, samples were fixed with 4% paraformaldehyde for 1 h and washed 3 times with PBS. After permeabilization for 30 min at RT with 0.2% Triton X-100 in PBS (Gibco), rhodamine phalloidin (Life Technologies, 1:200) and 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, 1 μg/mL) were added to the samples and incubated overnight at 4 °C with gentle rocking. Hydrogels were then extensively washed with several PBS changes and kept in PBS until imaging with an inverted Leica TCS SP5 confocal laser-scanning microscope.

Quantification of Cell Viability

The number of dead cells per field of view of the amnion was quantified from maximum Z intensity projections of stacks covering the full amnion thickness using Fiji from ImageJ [27, 28].

Quantification of Cellular Migration

Migration of cells into TG-PEG hydrogels was quantified using Fiji from ImageJ [27, 28]. The area drawn around the migrated cells (using the selection brush tool) was deducted from the area drawn around the original FM biopsy (using the freehand selection tool) (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000525559).

Statistical Analysis

As indicated in figure legends, *t* tests or two-way ANOVA with Tukey's multiple comparisons test were performed with GraphPad Prism (version 8.0.0): $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$, ns: nonsignificant.

Results

Human Amniotic Membrane Remains Viable upon 14 Days of in vitro Culture

To understand if the human amniotic membrane (Fig. 1a) remains viable when cultured in vitro, we collected FMs from planned cesarean deliveries and cultured them for up to 14 days. Confocal images of calcein- and ethidium homodimer (EthD-1)-stained amnion tissues (Fig. 1b) show a majority of alive cells, shown in green, for both the epithelium and the mesenchyme over the culture time. Dark areas within the cobble-stone-patterned epithelium indicate a slight loss of epithelial cells during culture. However, quantification of the dead cells

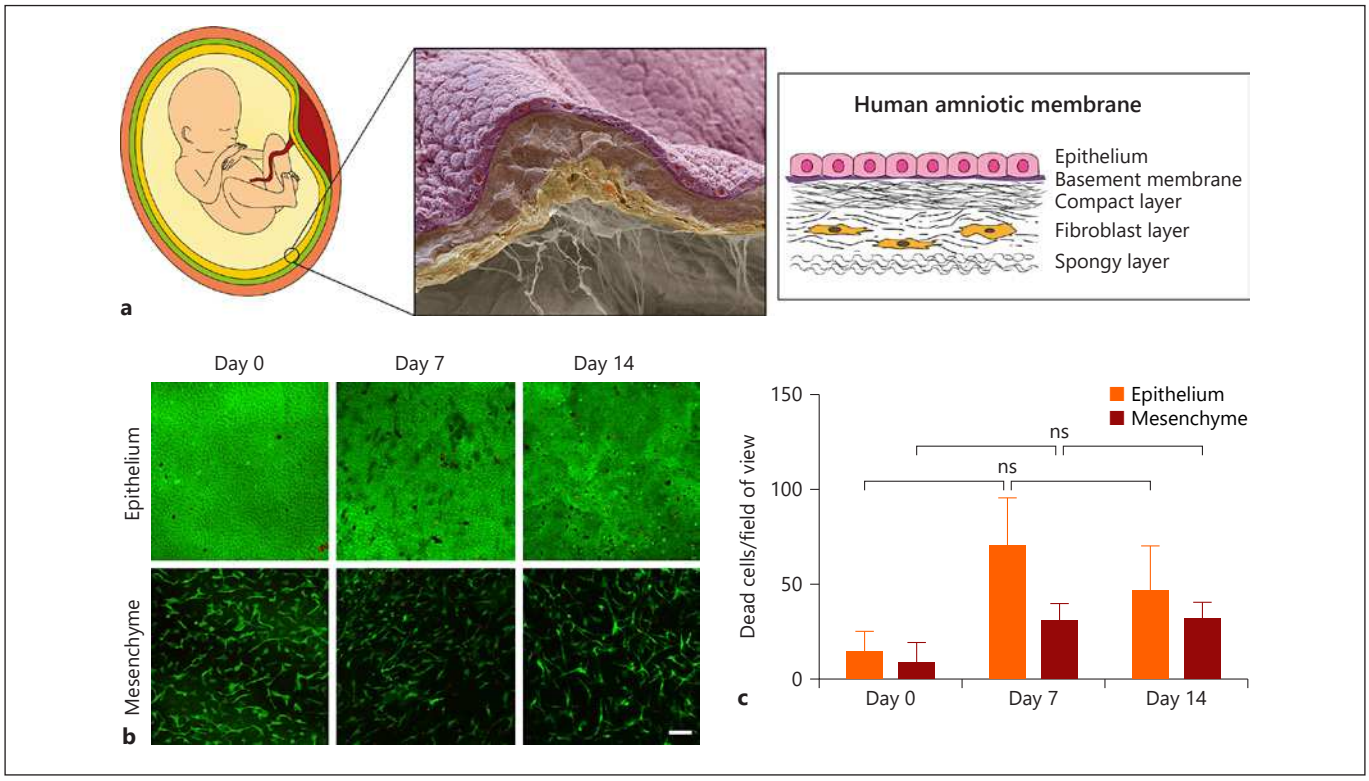

Fig. 1. FMs and their viability when cultured in vitro. **a** The amnion is the innermost layer of the FMs and is composed of 5 sublayers. Middle: Scanning electron microscopy image; scale bar, 25 μm. **b** Representative live (green: calcein AM)/dead (red: EthD-1) confocal images of the native amnion epithelium and mesenchyme over a 14-day culture period. Scale bar, 100 μm. **c** Quantification of dead cells per field of view in the amnion epithelium and mesenchyme over a 14-day culture period. Data are reported as mean ± SD. Two-way ANOVA with Tukey's post hoc correction. ns, nonsignificant; SD, standard deviation.

in the epithelium and mesenchyme revealed nonstatistical differences in the number of dead cells between day 0 and day 14 in culture (Fig. 1c), indicating that the amnion can be cultured and remains viable in vitro for at least 14 days.

Cell Viability Is Compromised in the Immediate Vicinity of Amnion Defects

During fetoscopies, the FMs are generally punctured with a 10-Fr trocar. This method of puncturing creates defects with variable shapes, which impedes standardized evaluations. To generate defects with reproducible and defined sizes in the FMs, we employed biopsy punches. Hematoxylin and eosin-stained histological sections through FM defects that were created with a 10-Fr trocar or with a 3-mm biopsy punch were not significantly different (Fig. 2). Live/dead staining of punctured amnions revealed a thin rim of dead cells in both the epithelium and the mesenchyme independently of the puncture method (Fig. 2). For this reason, a 3-mm biopsy punch was chosen as reproducible method for puncturing of amnion and full FM in the following experiments.

Ex vivo Model of Punctured Term FMs

Human amniotic mesenchymal stromal cells (hAMCs) were previously shown to migrate out of punctured amnion biopsies when cultured on tissue culture plastic (also termed "2D") [15]. Additionally, PDGF-BB stimulated the mobilization of hAMCs and their migration in TG-PEG biomaterials [24]. By combining these two observations, we aimed at exploring whether biomaterial-treated FM defects could heal in the presence of PDGF-BB. To do so, we used 3-mm diameter FM biopsies and created 1-mm holes in the center with a biopsy punch. The resulting disks were embedded in a TG-PEG hydrogel and cultured in media containing 100 ng/mL PDGF-BB (Fig. 3a). After 14 days of culture, we observed a mild cellular infiltration into the 1-mm puncture site. Surprisingly, however, a strong migration from the outer rim of the 3-mm biopsy into the TG-PEG hydrogel was observed (Fig. 3b).

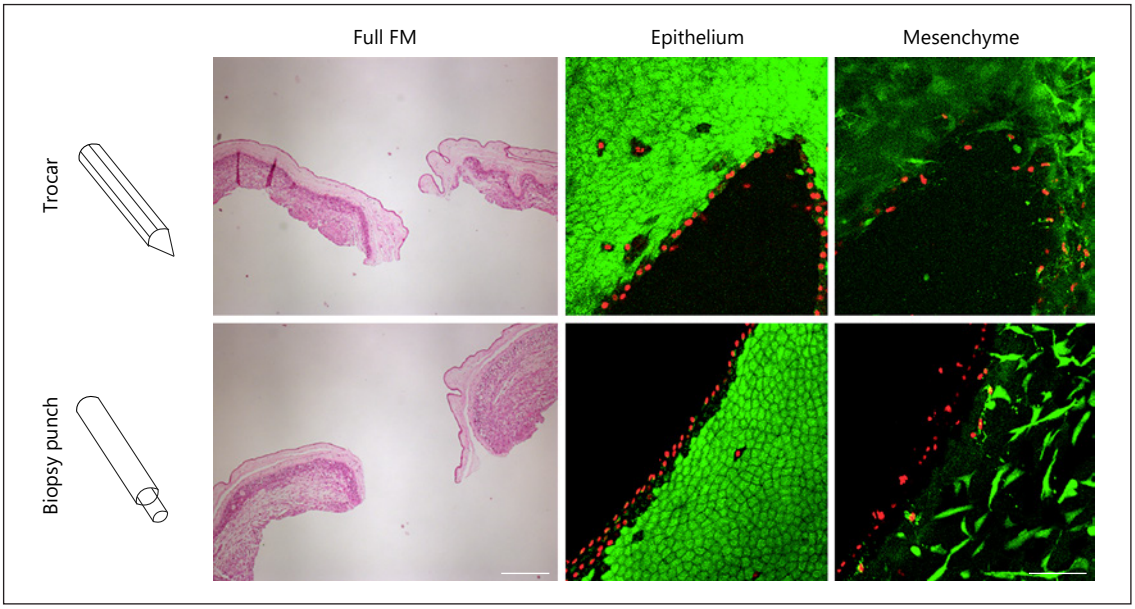

Fig. 2. Viability of FMs upon culture. A 10-Fr trocar and a 3-mm biopsy punch were tested to puncture the FMs. Middle: Hematoxylin and eosin staining of FMs punctured with a 10-Fr trocar or a 3-mm biopsy punch at day 0. Scale bar, 500 μm. Right: Representative live (green: calcein AM)/dead (red: EthD-1) confocal images of the native amnion epithelium and mesenchyme punctured with a 10-Fr trocar or a 3-mm biopsy punch at day 0. Scale bar, 200 μm.

While the inside migrated distances reached only ca. 50 μm, the outside migrated distances were up to 1.3 mm. Quantification of the migrated area revealed a clear superior migration toward the outside of the biopsy than into the 1-mm puncture site, however nonsignificant (Fig. 3c). Despite being a mild cellular infiltration, these results show that FM cells are able to migrate into an implanted biomaterial that bridges the defect, under the appropriate stimulating queues.

Miniaturized ex vivo Model Enables the Assessment of Amnion Cell Migration

Hence, to establish a model to study FM healing-promoting signals with a higher throughput and a very controlled tissue, we aimed at assessing the mobilization of cells from small amnion biopsies. We decided to focus on the amnion because it has been reported to be the major load-bearing structure [29], and spontaneous healing has been observed in the mouse in vivo model [30]. Remarkably, the amnion can be isolated as a clean transparent membrane and, contrary to the chorion, does not contain any attached decidua. To develop our system, we encapsulated 1-mm biopsy punches of term amnion tissue into TG-PEG hydrogels and stimulated them with 100 ng/mL PDGF-BB for 14 days (Fig. 4a). The area of cells from the amnion biopsy that infiltrated the biomaterial was superior under PDGF-BB stimulation, as shown by confocal imaging of their actin cytoskeleton (Fig. 4b, c). However, the cell-invaded areas did not reach significance.

Miniaturized ex vivo Model for Preterm Cell Migration

Treatments to promote the preventive healing of FM defects after fetal surgeries must be studied with tissues that closely resemble the traumatized tissue. Therefore, we collected small (typically 5–10 mm^2 sized) amnion biopsies of preterm FMs. According to our previously developed model for term FMs, we encapsulated 1-mm preterm amnion biopsies in TG-PEG hydrogels and cultured them in the absence or presence of 100 ng/mL PDGF-BB (Fig. 5a). After 14 days of culture, amnion cell migration was observed into TG-PEG hydrogels upon PDGF-BB stimulation, while amnion biopsies cultured in the absence of PDGF-BB showed no migration (Fig. 5b). Comparison of different donors revealed a relationship between GW and migration, with the earliest tissue showing a strong and significant migration under PDGF-BB stimulation (Fig. 5c).

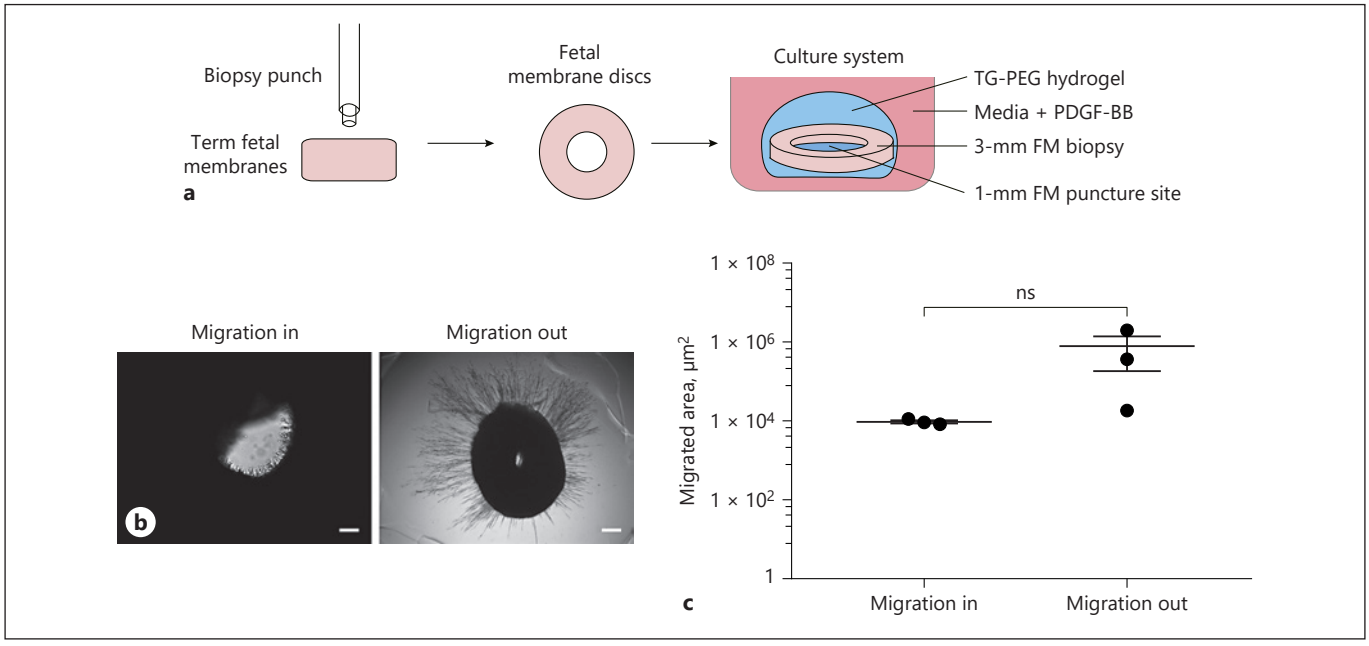

Fig. 3. Ex vivo model of punctured FMs. **a** Representation of the model. FM disks were prepared by puncturing a 3-mm FM biopsy with a 1-mm biopsy punch. FM disks were embedded in a TG-PEG hydrogel and cultured for 14 days under 100 ng/mL PDGF-BB stimulation. **b** Left: Representative image showing the migration of cells into a 1-mm puncture site filled with a TG-PEG hydrogel under 100 ng/mL PDGF-BB stimulation (100 ng/mL) after 14 days. Scale bar, 100 µm. Right: Representative image showing the migration of cells out of the 3-mm FM biopsy into a TG-PEG hydrogel under 100 ng/mL PDGF-BB stimulation after 14 days. Scale bar, 400 µm. **c** Quantification of cell migration into the defect or outside of the biopsy (both covered with TG-PEG hydrogel) under 100 ng/mL PDGF-BB stimulation (data are presented as mean values of $N = 3$ donors ± SEM). Unpaired two-tailed t test; ns, non-significant; SEM, standard error of the mean.

Discussion

In this study, we developed a model to investigate the healing of preterm amnion using an engineered and modularly designed biomaterial. This model requires only very small amnion biopsies and therefore enables the testing of multiple parameters affecting its healing under fully defined conditions.

First, we showed that during a 14-day in vitro culture both the amnion epithelium and the mesenchyme maintain a good viability. Puncturing the amnion with either a 10-Fr trocar or a 3-mm punch resulted in a similar thin rim of dead cells at the puncture site, which indicates that the biopsy punches can be used to create fetoscopy-mimicking FM defects in vitro. Our findings are in line with previous reports where the number of dead cells in the punctured area of FMs did not increase over time [17].

The aim of this study was to investigate 3D migration-based healing of preterm miniaturized amnion biopsies. We have earlier observed the GF-induced in vitro migration of amnion cells out of term human amnion fragments and isolated hAMCs, when encapsulated in TG-PEG hydrogels [24]. Here, the biomaterial-mediated healing of full FMs was first investigated with 3-mm biopsies that contained defined 1-mm defects, which were embedded in a TG-PEG hydrogel. Interestingly, in this model, we observed that cells migrated into the FM defect but migration from the outer rim of the FMs was much more pronounced. This effect could be due to differences in tissue tension at the outer compared to the inner rim, which in previous research has been described by collagen fibers arranging parallel to the axis of the wound edge [31, 32]. However, independent of potential effects driven by local mechanical properties, the outgrowth of cells from amnion biopsies is an easy amenable model to investigate novel migration-promoting signals and test potential healing-inducing biomaterials with a higher throughput and to verify their function with very limited quantities of preterm tissue specimens. Such assays will be important to further elucidate how the lack of healing of amnion defects is related to the local mechanical properties of the FMs at the wound edge, a lack of a healing-promoting scaffold, or even an active

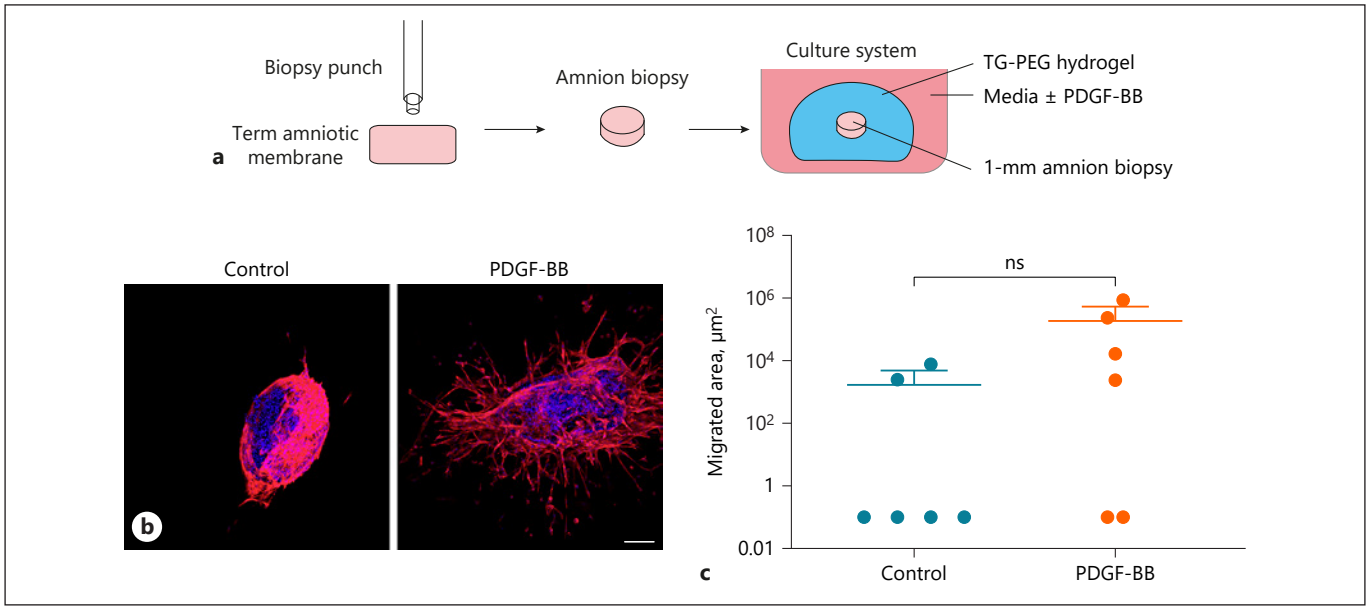

Fig. 4. Ex vivo model with term microtissues. **a** Representation of the model. A 1-mm biopsy is embedded in a TG-PEG hydrogel and stimulated, or not, with 100 ng/mL PDGF-BB. **b** Representative confocal images (actin is red, and nuclei are blue) showing the migration of cells into TG-PEG hydrogels in control media and under 100 ng/mL PDGF-BB stimulation after 14 days. Scale bar, 200 µm. **c** Quantification of cell migration into TG-PEG hydrogels from term amnion membranes (N = 6 donors). Data are presented as mean values of N = 6 donors ± SD). Unpaired two-tailed t test; ns, nonsignificant; SD, standard deviation.

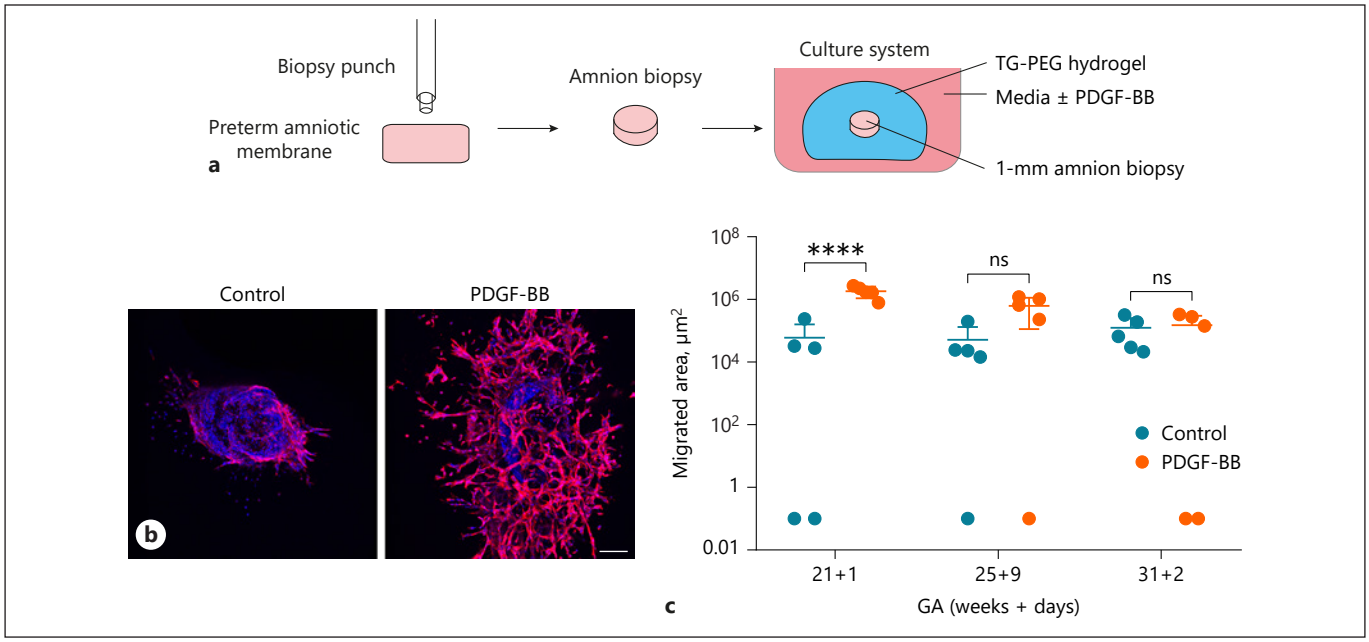

Fig. 5. Ex vivo model with preterm amnion microtissues. **a** Representation of the model. A 1-mm preterm amnion biopsy is embedded in a TG-PEG hydrogel and stimulated, or not, with 100 ng/mL PDGF-BB for 14 days. **b** Representative confocal images (actin is red, nuclei are shown blue) showing the migration of amnion cells into TG-PEG hydrogels in controls and under 100 ng/mL PDGF-BB stimulation after 14 days. Scale bar, 200 µm. **c** Quantification of preterm amnion cell migration into TG-PEG hydrogels under 100 ng/mL PDGF-BB stimulation (N = 3 donors, n = 5 per condition). Data are reported as individual data points ± SD. Unpaired two-tailed t test; ns, nonsignificant; SD, standard deviation.

growth inhibition. Furthermore, such ex vivo experiments are very necessary studies, since they contribute to the reduction of the number of animal experiments performed.

Since the FMs are dynamic tissues that evolve during pregnancy [33, 34], their response to biomaterials and signals is expected to be strongly dependent on the GW of pregnancy. Therefore, in this study, we aimed at using FMs representing the time of fetal intervention. During open *spina bifida* surgeries, very small (typically 5–10 mm^2) FM tissues emerge from the opening of the amniotic cavity, which makes using currently employed ex vivo amnion-healing models [15, 21] unfeasible. To test healing functions with such small FM tissue samples or to screen for new signals with an increased throughput, the encapsulation of small FM punch biopsies in biomaterials is a promising approach. To establish this model, we first used the more available term amnion to optimize the model with 3D encapsulated 1-mm biopsies and then applied this to preterm amnion. While our investigations confirm that PDGF-BB can promote the 3D migration of cells from term amnion, it indicates that there is a large donor-to-donor as well as site-to-site variability. In preterm samples with three different donors, we observed a trend towards a more pronounced amnion cell migration compared to term amnion, even in the absence of PDGF-BB. Interestingly, we observed a correlation between the GW of preterm samples and their response to PDGF-BB, with cells of the earliest midterm donor significantly migrating under PDGF-BB stimulation. These data confirm the earlier experiments performed in 2D with preterm cells, which described the migration-inducing GFs tumor necrosis factor alpha, fibrinogen, and PDGF-BB [18].

Here, we used an engineered PEG-based hydrogel, which by the modular assembly of defined components can be mechanically and biologically precisely tailored for the desired application [35–38], similar to other recently described synthetic biomaterials for the 3D culture and healing of FMs [21]. In comparison with engineered biomaterials, naturally occurring biomaterials such as collagen type I hydrogels comprise inherent properties, which on the one hand support cell functions in vitro and even healing of small FM defects in vivo [19], but on the other hand are difficult to control, resulting, for example, in too fast proteolytic degradation. Recently, various hybrid hydrogel systems have been described, enabling the integration of the advantages of synthetic and natural occurring biomaterials [35, 39, 40]. The model described here can be used to optimize and test the composition of such novel hybrid hydrogels with superior functions for both in vitro and later in vivo applications.

The future engineering of biomaterials and strategies for the preventive healing of the FMs after fetal therapies relies on robust in vitro models which enable to test the responses of native preterm FMs. This, to our knowledge, is the first miniaturized model to study 3D migration of preterm amnion cells into a bioengineered hydrogel to screen for healing-promoting or healing-inhibiting queues. Thanks to the minimal unspecific binding of proteins (such as staining antibodies) and the advantageous optical properties of TG-PEG, this model can easily be adapted for the elucidation of proliferation and extracellular matrix-inducing signals that additionally need to be explored for the development of next-generation FM healing biomaterials.

Conclusion

In this study, we developed a miniaturized model to study the healing potential of preterm amnion tissues. Furthermore, our work successfully demonstrates that PDGF-BB is able to stimulate migration from preterm small-scale tissues embedded in a fully synthetic engineered biomaterial. Employing our engineered model, the analysis of other healing-promoting or healing-inhibiting queues could be performed, in the path to understanding the reason for the lack of healing of the FMs after a fetoscopic intervention.

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Statement of Ethics

This study protocol was reviewed and approved by the Ethical Board of the Kanton of Zurich, approval number Stv07/2007 for term FMs and approval number PB 2020_00066 for preterm FMs. Written informed consent to participate was obtained from all participants involved in this study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Eva Avilla-Royo, Nicole Ochsenbein-Kölble, and Martin Ehrbar conceived the project. Flurina Famos and Eva Avilla-Royo collected the samples that Ladina Vonzun and Nicole Ochsenbein-Kölble provided. Flurina Famos, Eva Avilla-Royo, and Martin Ehrbar designed the experiments. Flurina Famos and Eva Avilla-Royo performed the experiments, and analyzed and interpreted the data. Ladina Vonzun, Nicole Ochsenbein-Kölble, and Martin Ehrbar supervised the project. All authors contributed to writing and manuscript revision.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further questions can be addressed to the corresponding authors.

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